ES 221 Problem Set 1

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1 Question 1: Molar Water Solubility of Betamethasone

We estimate the molar water solubility, S_W, of betamethasone, a common steroidal mediciation, using a standard equation:

$$\log_{10} S_W = 0.8 - 0.01(T_m - T) - \log_{10} P \tag{1}$$

The melting temperature, T_m, and log octanol-water partition coefficient, $\log_{10} P$, were obtained from the website *drugbank.ca*. These values were found to be $232^\circ C$ and 1.94, respectively, yielding a log molar water solubility of -3.21, or $S_W = 0.616mM$ at room temperature ($25^\circ C$), or $0.813mM$ at body temperature ($37^\circ C$). This is comparable to the reported literature value of $S_W = 0.60mM$, with the slight deivation likely due to measurement uncertainty or instrumental error, either in the measurement of the solubility itself or of the properties used in calculating the theoretical value. The somewhat low molar solubility of this compound is consistent with a balance of its high melting temperature (indicative of a high heat of fusion and stable crystalline form) and low octanol-water partition coefficient (despite the hydrophobic nature of steroids, owing to the presence of hydroxyl groups on the molecule).

2 Question 2: Molar Water Solubility of Candidate Drugs

We can now extend this analysis to three new candidate taxanes for disrupting microtubule activity in cancer cells. The compounds, $A36$, $A48$, and $A67$, have log octanol water partition coefficients of 1.5, 2.2, and 5.6, and melting temperatures of $343^\circ C$, $205^\circ C$, and $195^\circ C$, respectively. Through the same analysis and formula as in the previous question, we obtain molar water solubilities of $0.132mM$, $0.631mM$, and $0.3\mu M$, respectively. None of these reach the specified goal of $1mM$ water solubility, but as compound $A48$ has the largest solubility of the three, owing to its best balance between a low octanol-water partition coefficient and low melting temperature, it should be chosen for further analysis.

3 Question 3: Critical Micelle Concentration of Pacific1

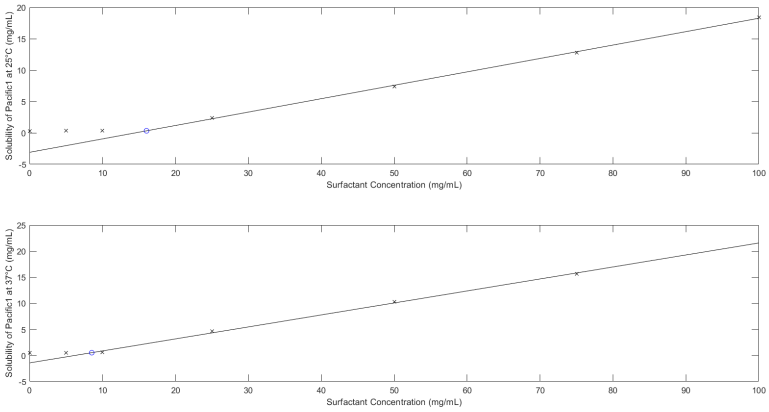


Figure 1: Solubility of Pacific1 as a Function of Surfactant Concentration at Room (top) and Body (bottom) Temperatures.

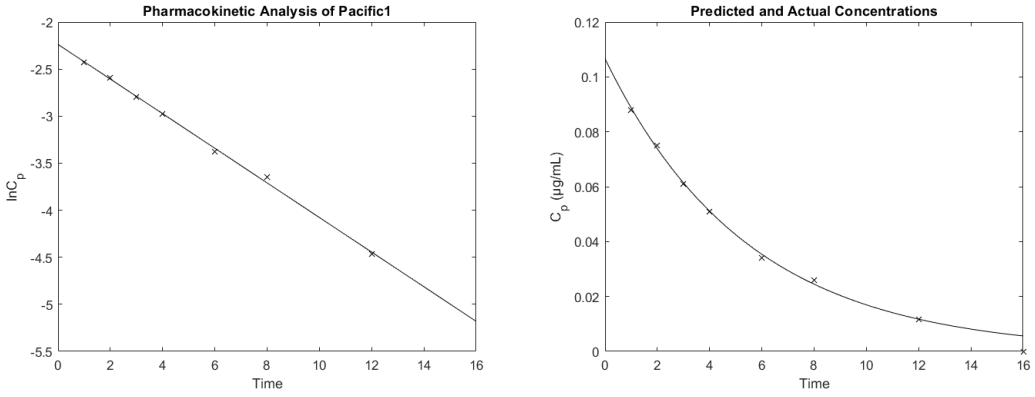
The compound Pacific1 (analogue $A48$ above) is chosen for further study based on the aforementioned considerations. Taking into account the observed molecular weight of the compound, $593Da$, the previously calculated water solubility is equivalent to $0.374mg/mL$ at room temperature. In order to develop a micellar delivery system, the solubility of Pacific1 was measured as a function of P2020 poloxamer concentration at both room and body temperature. The results are displayed in Figure 1, which also include trendlines for the linear dependence of solubility as a function of surfactant concentration above the critical micelle concentration (CMC), the lowest concentration needed for aggregation of monomers to form micelles. For the room temperature plot, it is clear from the fit that this linear regime does not begin until after the third data point. The water solubility in monomer is taken to be the average of the first three data points ($0.323mg/mL$, which is slightly below the theoretical value calculated above. Again, this may be due to measurement uncertainty, instrumental error, or impurities in the system. The CMC is

the surfactant concentration at which the calculated trendline (fit using linear regression in Matlab) is equal to the water solubility of the drug. This corresponds to a value of $16.0mg/mL$ at room temperature $25°C$.

At body temperature, $37°C$, this situation is a bit more complicated, as it is unclear if the third data point should be included in the linear regression dataset. Including the value to be part of the trend (i.e. assuming the value of $10mg/mL$ is above the CMC) yields a CMC of $8.53mg/mL$, while not including the value actually decreases the CMC to $6.21mg/mL$. It is difficult to say whether this third point should be included, as the corresponding solubility of Pacific1 may be below the trendline simply due to experimental uncertainty, or it may be that this is still below the true CMC. In order to be conservative, we choose the larger value of the CMC, $8.53mg/mL$, including the third data point in the trendline.

Our requirements are to select a concentration of surfactant (P2020) such that the corresponding solubility of Pacific1 is larger than $10mg/mL$. Since this criterion does not specify a temperature, we assume this corresponds to the room (processing) temperature. If we restrict ourselves to experimental surfactant concentrations, this only leaves us with $75mg/mL$ and $100mg/mL$ data points. As the latter does not have a corresponding solubility at body temperature, we should not choose this value as we cannot be certain how the micellar system will function beyond the $75mg/mL$ data point. Therefore, we select a poloxamer concentration of $75mg/mL$ to exceed the room temperature solubility of $10mg/mL$. As the solubility is found to increase as a function of increasing temperature, precipitation is not expected upon infusion, since precipitation requires a decrease in solubility.

4 Question 4: Bolus Pharmacokinetics of Pacific1



(a) Log-linear. (b) Linear.

Figure 2: Solubility of Pacific1 as a Function of Surfactant Concentration at Room (top) and Body (bottom) Temperatures.

In order to examine the pharmacokinetic properties and dose-limiting toxicity of Pacific1, data from a Phase I clinical trial in response to an IV bolus of dose $10mg$ were plotted on a log-linear scale of plasma concentration vs. time (likely in hours but units are not specified). The lack of a noticeable distribution phase on this log-linear plot suggests the applicability of a one-comparment model, of the form:

$$\ln C_p = \frac{D}{V_1} - k_e t \tag{2}$$

In this equation, D is the delivered dose ($10mg$), V_1 is the distribution volume, and k_e is the elimination rate constant, related to the elimination half life by $t_{1/2} = \ln 2/k_e$. Fitting the data to this one-compartment model yields fit parameters of $V_1 = 93.8L$ and $k_e = 0.184hr^{-1}$, or equivalently, $t_{1/2} = 3.77hr$. The second figure above shows the linear-linear fit of the model to the experimental data, demonstrating the correct ability of the model to predict the experimental plasma concentration. This is further evidenced by the sum of squared errors (SSE) of the log-linear fit, which is found to be only $0.0059\mu g^2/mL^2$. The large volume of distribution (much larger than the plasma volume of $3L$) suggests that the drug rapidly distributes to the periphery at this concentration, a prediction that is further confirmed by the short half-life and rapid elimination rate constant. It should be noted that the final time point is excluded from the fitting procedure as the value is below quantification.

5 Question 5: Zero-Order Pharmacokinetics and Pharmacodynamic Response of Pacific1

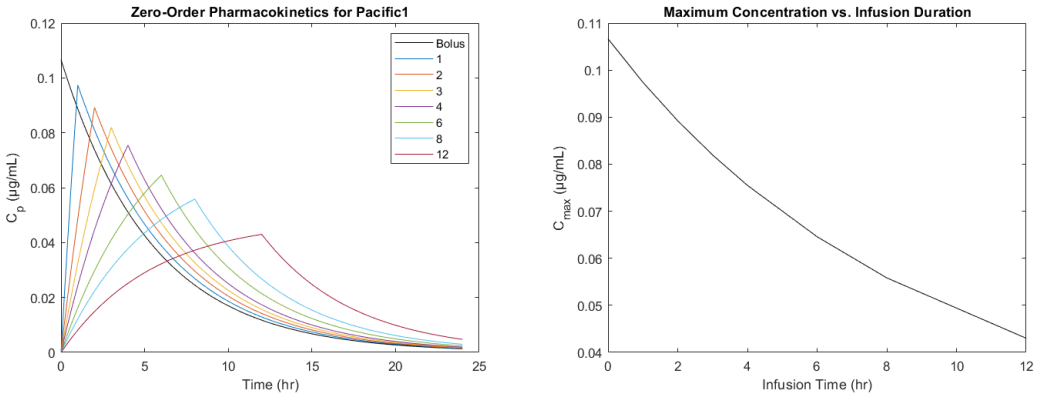
Rather than only consider the pharmacokinetic response to a bolus dose, we can also consider how the maximum plasma concentration, C_{max}, evolves as a function of infusion time using a zero-order infusion model. This analysis is especially relevant owing to the apparent dependence of chemotherapy-induced peripheral neuropathy (CIPN) on the maximum plasma concentration obtained throughout the duration of the infusion. We can model the dynamics of a zero-order infusion using the following equations:

$$C_p = \frac{D}{t_{inf} V_1 k_e}(1 - e^{-k_e t}) \tag{3}$$

and

$$C_p = \frac{D}{t_{inf} V_1 k_e}(1 - e^{-k_e t_{inf}}) e^{-k_e (t - t_{inf})} \tag{4}$$

Here, t_{inf} is the infusion time, which we model between 1 hour and 12 hours (specifically, 1, 2, 3, 4, 6, 8, and 12 hour infusions), and the other parameters are the same as defined and found from the bolus injection. It is apparent from the equations that the maximal achieved plasma concentration is obtained at the final time point of the infusion, after which the concentration begins to decrease exponentially. Thus, $C_{max} = \frac{D}{t_{inf} V_1 k_e}(1 - e^{-k_e t_{inf}})$. We can further confirm this by plotting the plasma concentration profiles (Figure 3a), which yield the relationship between maximal plasma concentration and infusion time displayed in Figure 3b. Clearly, longer infusion times with the same total dose reduce the maximal achieved plasma concentration which, according to the aforementioned relationship between C_{max} and CIPN, should reduce the adverse effects of the drug. Subsequent data were obtained from bolus and 1-



(a) Plasma Concentration.

(b) Maximal Concentration vs. Infusion Time.

Figure 3: Zero-Order Pharmacokinetic Analysis.

to 6-hr infusion duration trials with 8 patients in each group. The modified Total Neuropathy Score (mTNS) was assessed at the begin of the study and at the end of a six-week trial after dosing for the prescribed time each day. The data are suggestive of an E_{max} pharmacodynamic response model, of the following form:

$$Effect = E_0 + \frac{E_{max} C_{max}^n}{EC_{50}^n + C_{max}^n} \tag{5}$$

The model parameters are the initial level of CIPN, as assessed by the initial mTNS score, which is averaged over the six treatment groups and found to be $E_0 = 2$. The other three parameters were estimated based on the obtained data points, maximal effect $E_{max} = 13$, concentration for half-maximal response $EC_{50} = 0.065 \mu g/mL$, and Hill exponent $n = 5$. These initial estimates were used with the obtained data as inputs to Matlab's nonlinear regression function, $fitnlm$. The result yields a model fit with $p = 4.37e-5$ and an adjusted $R^2 = 0.963$, suggesting good predictability and accuracy with respect to experimental data. The following figure shows the model prediction and the experimental results. Based on these data, we obtain final values of the model parameters of $E_{max} = 12.2$, $EC_{50} = 0.0701 \mu g/mL$, and $n = 5.93$. Given that the lowest maximal concentration, obtained from a six-hour infusion time, was only $0.006 \mu g/mL$ below the half-maximal concentration, there is clearly room for further improvement. Prolonged infusion times should lead to lower maximal plasma concentrations, and based on the results of this model, substantially reduced mTNS scores at six weeks.

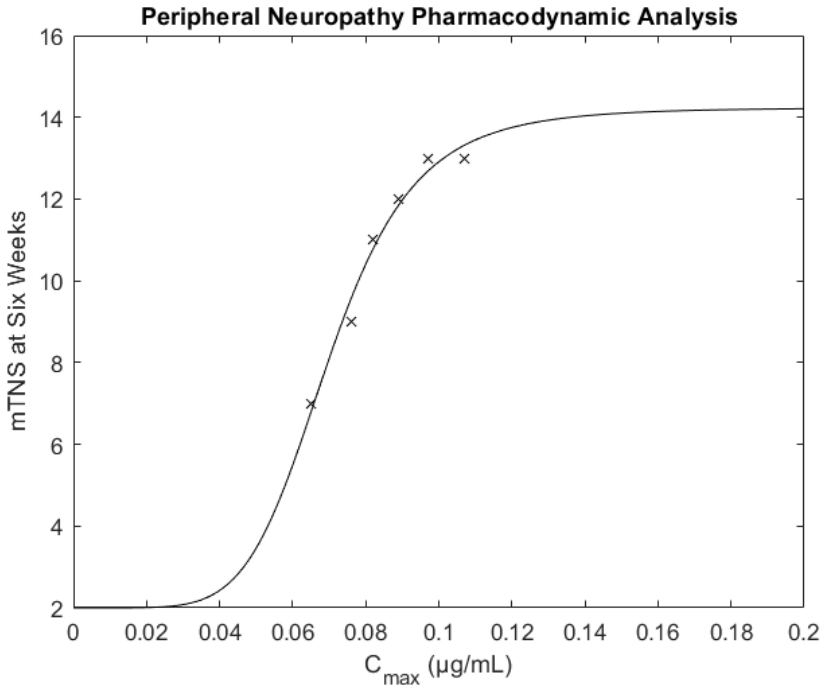


Figure 4: Pharmacodynamic Analysis of Maximal Plasma Concentration and CIPN at Six Weeks of Treatment.

6 Question 6: Optimization of PEG and Pacific1 Concentrations for Nanoparticle Delivery

A separate drug delivery vehicle that can be used to deliver Pacific1 is a passively targeted nanoparticle, which is expected to accumulate in the tumor due to the enhanced permeation and retention (EPR) effect, at least to some degree, as a consequence of fenestrations in the tumor vasculature and decreased lymphatic activity in the tumor. Encapsulating the drug in a nanoparticle is expected to reduce the CIPN levels produced by the micellar delivery system, allow for shorter delivery time, and aid treatment of various cancers. Optimization of poly(lactide-co-glycolide) (PLGA) molecular weight and lactide/glycolide ratio has already been performed, and we now seek to optimize the fraction of PLGA which is covalently bound to poly(ethylene glycol) (PEG) 5000 and the drug load (weight fraction) in the nanoparticle to faciliate a 12-to-24 hour release profile for a 10 mg total dose. Six combinations of these parameters were evaluated, with weight fractions of 0.05, 0.10, and 0.20 Pacific1, and PLGA-PEG ratios of 0.1 and 0.2 (i.e. every tenth or every fifth PLGA is covalently bound to a PEG chain). The resulting plasma concentration in $\mu g/mL$ vs. time plots highlight the role of each of these parameters in controlling the pharmacokinetics of the drug delivery system. The inclusion of PEG serves to shield the nanoparticle from the mononuclear phagocyte system (MPS), which is the main source of clearance of these nanoparticles. Without a sufficient concentration of PEG on the surface, each molecular chain will curl in on itself (the so-called "mushroom" model), and be unable to prevent phagocytosis and subsequent degradation by the immune cells. Larger concentrations of PEG form a "brush border" on the surface of the particle, effectively masking it from the MPS and substantially prolonging circulation time. As a result, this gives the particles a higher probability of ultimately extravasating into the tumor tissue. Therefore, the higher PEG fraction of 0.20 is desirable. Of course, it should be noted that side effects must still be monitored before this higher level is definitively chosen. The levels of drug are well above those found to cause CIPN in the micellar formulation, but given that these concentrations reflect both encapsulated and free drug (the former of which should be much larger), it is expected that the levels of free drug available should be much lower and cause a substantial reduction in observed CIPN.

In choosing a weight fraction of the drug, we can consider the shape of the release curves. Weight fractions larger than 0.05 show a pronounced distribution phase, likely resulting from burst release of the drug upon infusion. This burst can be caused by several factors, including, but not necessarily limited to, drug being incompletely encapsulated by the nanoparticles due to concentrations exceeding the solubility of the drug in the PLGA particles, or the presence of the drug changing the conformation or phase of the nanoparticles, and as a result, the release kinetics of the drug

once in the vasculature. In any case, this will cause spikes in the plasma concentration just after infusion, increasing the likelihood of adverse side effects. Therefore, we would preferably select a weight fraction of 0.05 to prevent this burst release. An alternative method would be to try to enhance the solubility in the particles to allow for larger drug loading concentrations, but based solely on the available data, the lowest concentration is the best.

7 Appendix

Script to evaluate room temperature molar water solubility of betamethasone and the three taxane analogues.

```
l10Sw = @(Tm,T,lP) 0.8-0.01*(Tm-T)-lP;

T = 25;

Tmbms = 232;          %from drugbank.ca
lpbms = 1.94;
lSwbms = l10Sw(Tmbms,T,lpbms);
Swbms = 10^lSwbms;

Tm = [343,205,195];
lp = [1.5,2.2,5.6];
MW = [633,593,621];
Sw = zeros(1,3);

for i = 1:3
    lSw = l10Sw(Tm(i),T,lp(i));
    Sw(i) = 10^lSw;
end

Swg = Sw.*MW;
```

Script to determine critical micelle concentrations at room temperature and body temperature by equating the average solubility below the CMC to the predicted trendline beyond the aggregation limit.

```
CsurfTrial = [0;5;10;25;50;75;100];
Sol25 = [0.31;0.34;0.33;2.4;7.4;12.8;18.4];
Sol37 = [0.52;0.53;0.55;4.6;10.3;15.6];

figure;
subplot(2,1,1);
plot(CsurfTrial,Sol25,'xk','LineStyle','none');
xlabel('Surfactant Concentration (mg/mL)');
ylabel(['Solubility of Pacific1 at 25', char(176), 'C (mg/mL)']);
p25 = polyfit(CsurfTrial(4:end),Sol25(4:end),1);
Csurf = [0:0.1:100];
Sol25model = p25(1)*Csurf+p25(2);
hold on;
plot(Csurf,Sol25model,'k');
Sw25 = mean(Sol25(1:3));
CMC25 = (Sw25-p25(2))/p25(1);
plot(CMC25,Sw25,'ob');
hold off;

subplot(2,1,2);
plot(CsurfTrial(1:end-1),Sol37,'xk','LineStyle','none');
xlabel('Surfactant Concentration (mg/mL)');
ylabel(['Solubility of Pacific1 at 37', char(176), 'C (mg/mL)']);
p37 = polyfit(CsurfTrial(3:end-1),Sol37(3:end),1);
Sol37model = p37(1)*Csurf+p37(2);
hold on;
plot(Csurf,Sol37model,'k');
Sw37 = mean(Sol37(1:2));
CMC37 = (Sw37-p37(2))/p37(1);
plot(CMC37,Sw37,'ob');
hold off;
```

Script to fit plasma concentration data to a one-compartment pharmacokinetic bolus model through the use of logarithms and linear regression. The SSE is also calculated for the model, and plots of the log-linear and linear-linear predictions and experimental data are constructed.

```
t = [1:4,6,8,12,16];
Cp = [0.088,0.075,0.061,0.051,0.034,0.026,0.0115,0];
D = 10;

figure;
plot(t,log(Cp),'xk','LineStyle','none');
xlabel('Time');
ylabel(['lnC_p']);
title('Pharmacokinetic Analysis of Pacific1');
hold on;

[fit,p] = polyfit(t(1:end-1),log(Cp(1:end-1)),1);
time = [0:0.1:16];
modellogCp = fit(1)*time+fit(2);
SSE = 0;
for i = 1:length(Cp)-1
    SSE = SSE + (fit(1)*t(i)+fit(2)-log(Cp(i)))^2;
end
plot(time, modellogCp,'k');
hold off;
V1 = D/exp(fit(2));
ke = -fit(1);
t12 = log(2)/ke;

figure;
plot(t,Cp,'xk','LineStyle','none');
hold on;
modelCp = (D/V1)*exp(-ke*time);
plot(time,modelCp,'k');
xlabel('Time');
ylabel(['C_p (',char(181),'g/mL)']);
title('Predicted and Actual Concentrations');
```

Script for plotting the pharmacokinetics of zero-order infusions of various durations up to a maximum of 24 hours beyond the start of the infusion. The trends are plotted along with the bolus dose kinetics, and the maximum plasma concentration is plotted as a function of infusion duration.

```
tinf = [1,2,3,4,6,8,12];
D = 10;
num = length(tinf);
R = zeros(num,1);
ke = 0.1839;
V1 = 93.7881;
tmax = 24;
ttot = 0:0.01:24;
tlength = length(ttot);
Cp = zeros(num+1,tlength);
Cmax = zeros(num+1,1);
Cp(1,:)=(D/V1)*exp(-ke*ttot);
Cmax(1) = (D/V1);
figure;
plot(ttot,Cp(1,:),'k');
hold on;
for i = 1:num
    R(i) = D/tinf(i);
```

```
    ton = 0:0.01:tinf(i);
    Cp(i+1,1:length(ton)) = (R(i)/(V1*ke))*(1-exp(-ke*ton));
    toff = [tinf(i)+0.01:0.01:tmax];
    Cp(i+1,(length(ton)+1):tlength) = Cp(i+1,length(ton))*exp(-ke*(toff-tinf(i))
        );
    plot(ttot,Cp(i+1,:));
    Cmax(i+1) = Cp(i+1,length(ton));
end
title('Zero-Order Pharmacokinetics for Pacific1');
xlabel('Time (hr)');
ylabel(['C_p (',char(181),'g/mL)']);
legend('Bolus','1','2','3','4','6','8','12');
hold off;

figure;
plot([0,tinf],Cmax,'k');
xlabel('Infusion Time (hr)');
ylabel(['C_{max} (',char(181),'g/mL)']);
title('Maximum Concentration vs. Infusion Duration');
```

Script for nonlinear regression of an E_{max} pharmacodynamic response model for the mTNS scores as a function of maximum plasma concentration.

```
E0 = mean([2,2,3,1,2,2]);
Cmax = [0.107,0.097,0.089,0.082,0.076,0.065];
mTNS = [13,13,12,11,9,7];
plot(Cmax,mTNS,'xk','LineStyle','none');

E = @(coeff,C) E0+coeff(1)*C.^coeff(2)./(coeff(3).^coeff(2)+C.^coeff(2));
cf = fitnlm(Cmax,mTNS,E,[13, 5, 0.065]);

Emax = cf.Coefficients.Estimate(1);
n = cf.Coefficients.Estimate(2);
EC50 = cf.Coefficients.Estimate(3);

Cmodel = 0:0.001:0.200;
Effect = E0 + Emax*Cmodel.^n./(EC50^n+Cmodel.^n);
Epredict = E0 + Emax*Cmax.^n./(EC50^n+Cmax.^n);
hold on;
plot(Cmodel, Effect,'k');
xlabel(['C_{max} (',char(181),'g/mL)']);
ylabel('mTNS at Six Weeks');
title('Peripheral Neuropathy Pharmacodynamic Analysis');
hold off;
```